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Technical Support Package

Using Spider-Web Patterns to Determine Toxicity

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Technical Support Package

for

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Using Spider-Web Patterns to Determine Toxicity

A. GENERAL PURPOSE

An apparatus and method for using statistical crystallography to determine and classify drugs by their chemical toxicity has been constructed. The method utilizes the sensitivity of the house spider, *Araneus diadematus*, to chemicals. This appears in the web of the spider. The web can be classified in one of five categories of toxicity.

B. PRIOR ART AND PREVIOUS METHODS

Experimental methods using animal testing are diminishing. According to Guy Murchie, the unique aspect about all drug-affected webs is that they so clearly characterize the drug that research laboratories have started domesticating spiders in order to test drugs. They have no method of determining quantitatively differences between webs. This study presents a way of doing so using statistical crystallography.

C. DISADVANTAGES OF PRIOR ART

Existing whole animal procedures are expensive, time-consuming and increasingly restricted by federal law. Researchers have considered breeding spiders for drug testing, but they have no way of quantitatively determining the effects of the drug.

D. COMPONENT PART IDENTIFICATION AND MODE OF OPERATION

Spiders raised specifically for testing will be injected with a chemical or drug. The control web is a normal web spun by the spider without the influence of any chemicals. The next web that the spider spins should be photographed. The drug-affected web can then be classified by judging the number of completed sides by comparison to the control web. The five categories are: 0 NCs, 1 NC, 2 NCs, 3 NCs, 4 NCs, and 5 NCs. NC stands for not completed sides. The toxicity of a chemical appears in the disorientation of the web. This makes the analyzing of the web a valid test. The four drugs that were used include: marijuana, caffeine, benzedrine, and chloral hydrate.

E. ALTERNATE EMBODIMENTS

Applications include enlarging the scope of drugs applied to include representative examples of carcinogens, insecticides, fungicides, petroleum products and organics, antimetabolites, and heavy metals. It could be used (to test disinfectants) in clinical, environmental, food and beverage, pharmaceutical, and cosmetic tests.

F. ADVANTAGES OVER PRIOR ART

Advantages of using statistical crystallography include:

1. A quantitative way to classify the toxicity of the drug.
2. Less expensive and less harmful alternative to animal testing.
3. Easier than existing methods and requires only photographic equipment.
4. More reliable than existing methods; the rank order of toxicity for spiders compares favorably with the known rank order of toxicity in rats.

G. NEW FEATURES

No classification of drug-affected spider webs has been attempted. The method proposed here presents a quantitative way to do so both inexpensively and easily. The accuracy of using spider webs to test chemical toxicity has been proved by comparing the results of a web test with those of known rat toxicities involving the same chemicals.

Additional information is provided in Attachment A, Statistical Crystallography: A Practical Method to Quantify Toxic Effect on Spider Web Patterns.

Statistical Crystallography: A Practical Method to Quantify Toxic Effect on Spider Web Patterns

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ABSTRACT

The statistics of random cellular patterns in five spider webs are analyzed to detect chemical effects. The average area, perimeter, and radius are determined for both the networks as a whole and each cell type. Agreement is found with a variety of topological relations previously found for other networks, namely Lewis's law and the Perimeter law. Despite the diverse underlying physics, web lattices are shown to share a broad class of geometric properties with other random, but physically unrelated, networks--metal grains, soap foams, bioconvection, and Langmuir monolayers. Here we quantify chemical toxicity using spider web patterns as the indicator; rank order listings for pharmaceuticals (benzedrine, caffeine, chloral hydrate, THC) compare favorably with previous rat toxicity ratings.

I. INTRODUCTION

One of the best known examples of animal communication is the spider web. Spiders use it to communicate with any creature that passes by, from victims to neighbors. The spider quickly spins this web in the early hours of the day using touch rather than sight. A new rung is added every second, leading to completion of a web within half an hour^{1,2}.

There are several types of webs, each of which represents but one of the thousand cobweb designs or dialects that have evolved in the last hundred million years. The patterns for spider webs are present in the genes of the spiders, yet the webs serve as a physical representation of the spiders' moods. A change in the environment, such as weather or food, influences the next web. An additionally powerful factor is a drug that the spider may recently have absorbed. The effects of this drug appear in the next web that the spider spins. The unique aspect about all drug-affected webs is that they so clearly characterize the drug that research laboratories have started domesticating spiders for the sole purpose of using their webs to test drugs¹.

Spider webs are an example of a cellular network similar to soap foams³, Langmuir monolayers⁴, and bioconvection⁵. These two-dimensional space-filling networks exhibit certain geometric properties⁶. We have taken the webs of spiders¹ which have been affected by caffeine, chloral hydrate, marijuana, and benzedrine. A "perfect" web spun on the ground was taken as control; the web also served as control for a previous Skylab experiment² testing the low gravity behavior of spider type *Araneus diadematus*. These webs are obviously different (Figure 1). By applying the techniques of statistical crystallography⁶, we hope to put these differences in quantitative terms which can then be later used by researchers as a tangible, exact way to differentiate the effects of various drugs and pharmaceuticals on spider webs.

II. EXPERIMENTAL PROCEDURE AND RESULTS

The five spider webs are shown in Figure 1. Four of the webs were affected by the drugs of marijuana, benzedrine, caffeine, and chloral hydrate. Each drug produces an effect which differs uniquely from the control. A caffeine web appears as a loose, ragged array of crooked, unfinished spokes. A benzedrine web displays more organization but less symmetrical balance. A marijuana web lacks outer spokes. A web spun under chloral hydrate is barely begun before the spider loses motor control¹.

Figure 2 shows a comparison of number of cells, average area, average radius, and average perimeter for the five web types. These averages were obtained using the ImageAnalyst program (Visicon, CA). The results appear consistent for all five webs. As the number of cells increase, the average area, radius, and perimeter of the membrane networks decrease.

The marijuana, benzedrine, and caffeine webs all displayed six-sided cells. The control and chloral hydrate webs did not, thereby defying Euler's theorem of hexagons as ideal space-filling cells. Due to the method which a spider employs when creating a web, the center cell is uniquely formed, with an unusually large n compared to the rest of the web. For this reason the center cells were excluded from the data analysis.

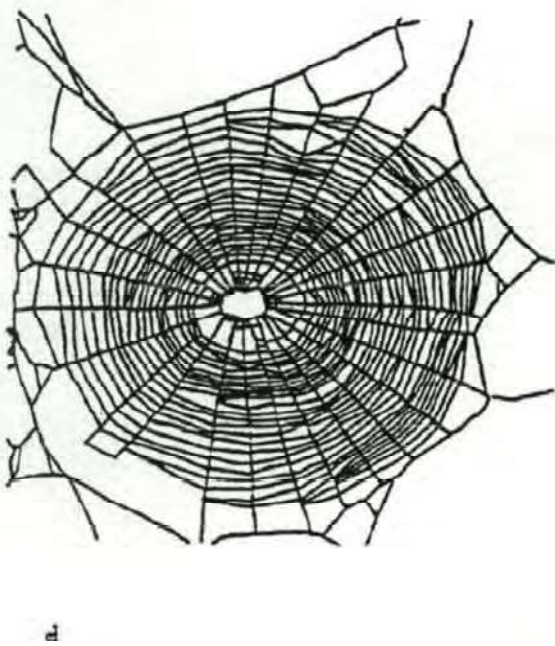


Figure 1
The five selected spider webs: a) Control, b) Marijuana, c) Benzodrine, d) Caffeine, and e) Chloral Hydrate.

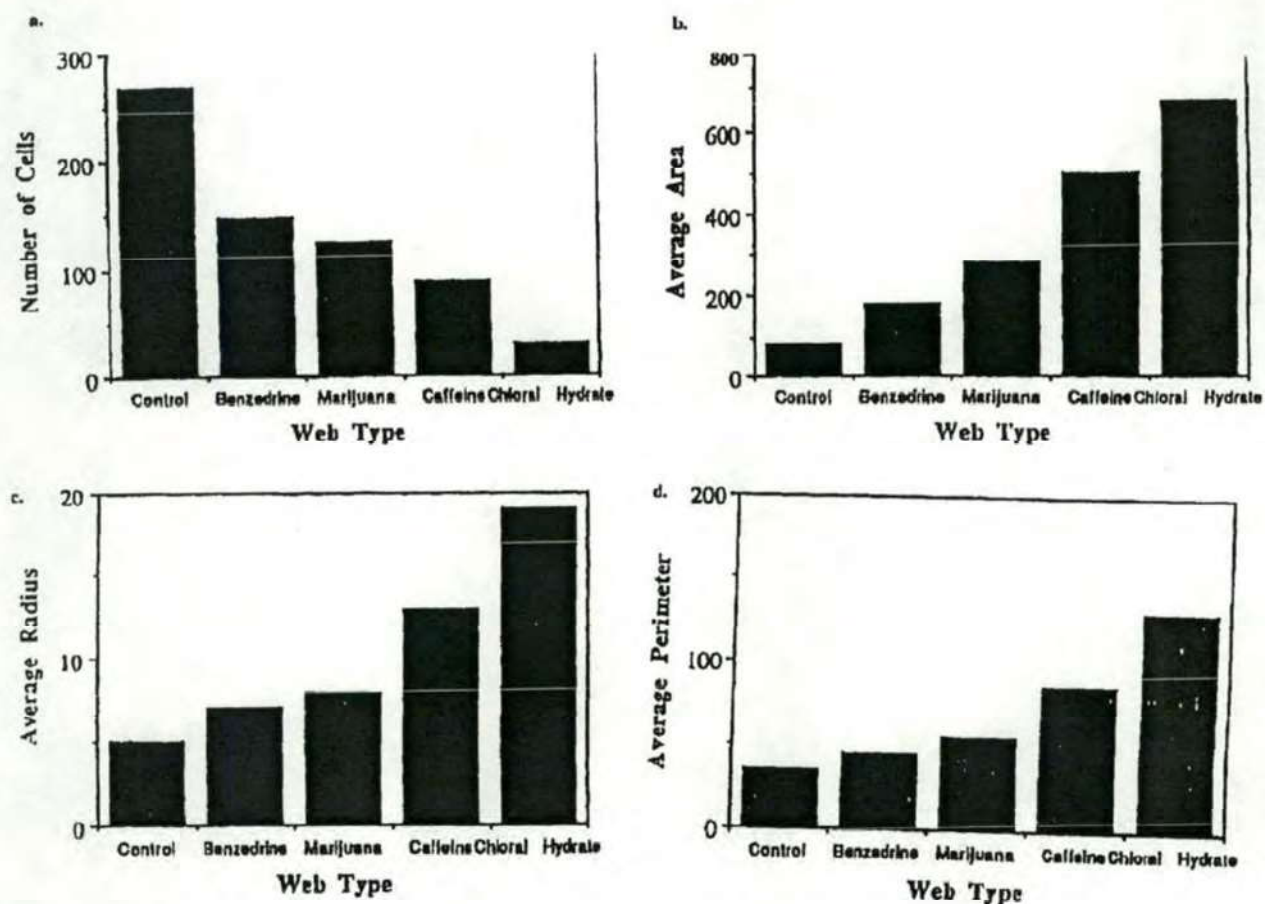


Figure 2

Comparisons between the five web networks of: a) Number of cells, b) Average Area, c) Average radius, and d) Average perimeter.

III. RESULTS OF STATISTICAL CRYSTALLOGRAPHY

The average area of n-sided cells was determined in arbitrarily chosen units. The data was obtained by randomly choosing representative cells with different numbers of sides. For example, six four-sided cells were chosen and their area was averaged. Figure 3 displays the adherence of all five webs to Lewis's Law¹. The correlation for all five webs was equal to or greater than 0.9.

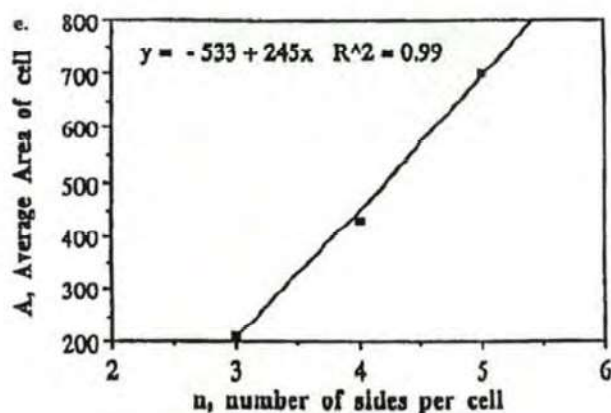
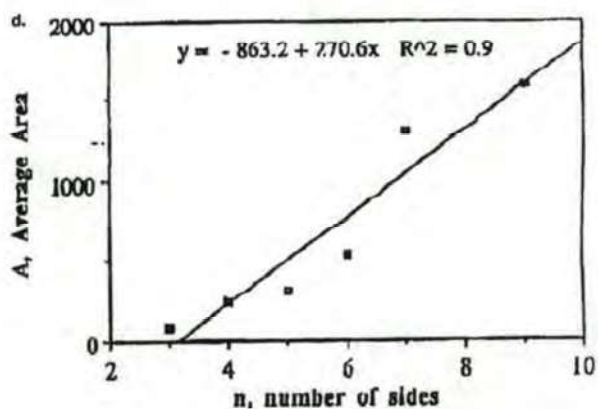
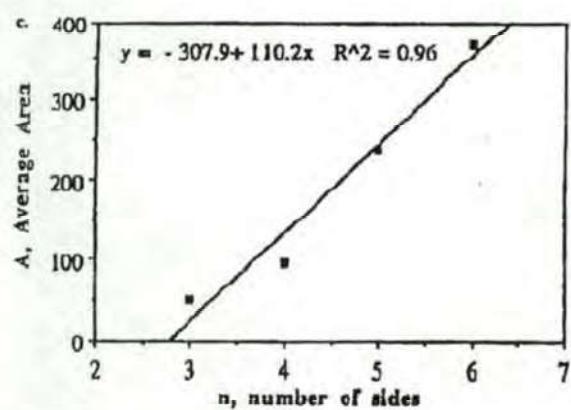
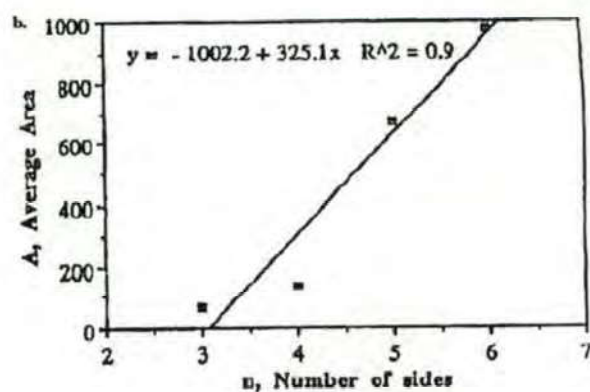
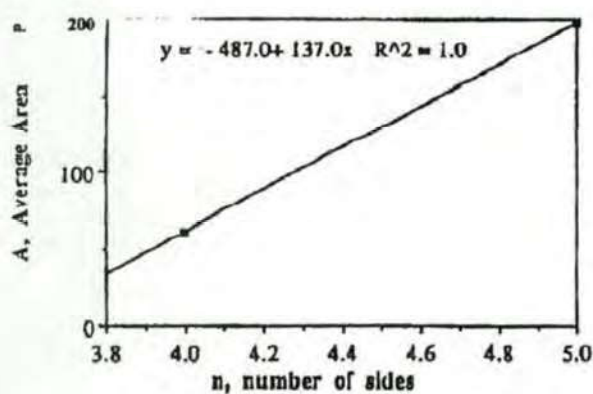


Figure 3

Average area as a function of the number of sides for each n sided cell in the: a) Control web, b) Marijuana web, c) Benzedrine web, d) Caffeine web, and e) Chloral web.

Lewis's Law implies a linear relationship between the average area of a cell and the number of sides of a cell. This is somewhat unexpected considering the also linear relationship with the average radius and the other analysis of vegetable cell patterns⁷ find a similar simultaneous agreement between a perimeter and area correlation. The average radius versus the number of sides appears in Figure 4. The webs of control, marijuana, benzedrine, and caffeine all showed a correlation equal to or greater than 0.9. Chloral hydrate had a correlation of 0.6, a result attributable in part to the smaller number of sample web polygons.

Figure 5 shows the average perimeter as a function of the number of sides of a cell. The data was obtained by a method similar to the one used in Figures 3 and 4. Representative cells were chosen and grouped according to the number of sides. The average perimeter was found for each set. The figure verifies the Perimeter Law for the control, marijuana, benzedrine, and caffeine webs. All these have a correlation equal to or greater than 0.9. The exception is the chloral hydrate web, with a correlation of 0.8. The Perimeter Law implies that energy is carried along the cell boundaries rather than the interior. This is consistent of a web design which depends on surface tension along an extruded fiber. The difference with the chloral hydrate web in the results for Figures 4 and Figure 5 may be explained by the fact that the spider never received a chance to finish its web; the drug caused it to lose motor control before it had the chance.

IV. TOXICITY

This study presents a simple way to classify drugs into categories according to their toxicity. Each web can be circumscribed by a rectangle. This rectangle can be used to specify four directions of the web: up, down, left, and right. When affected by a drug, the web is incomplete for at least one of the four sides. Table 1 shows which sides are complete for each of the five webs.

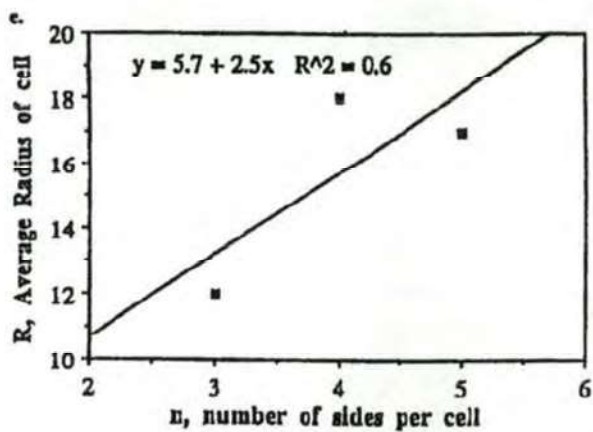
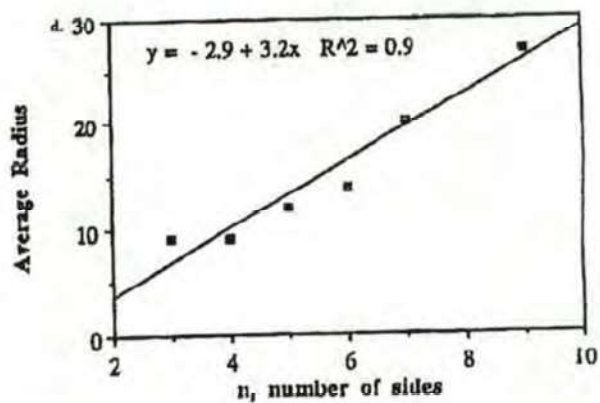
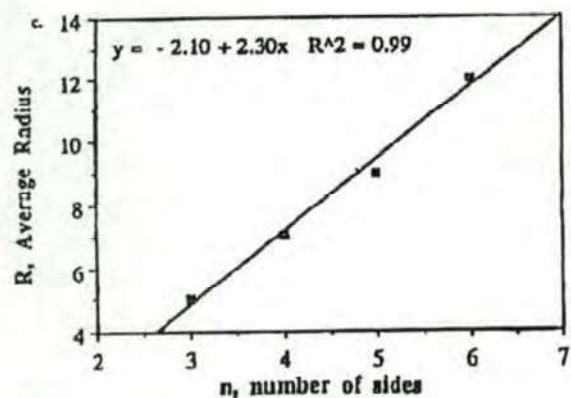
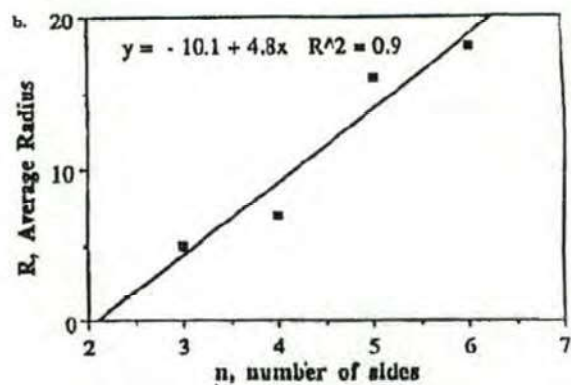
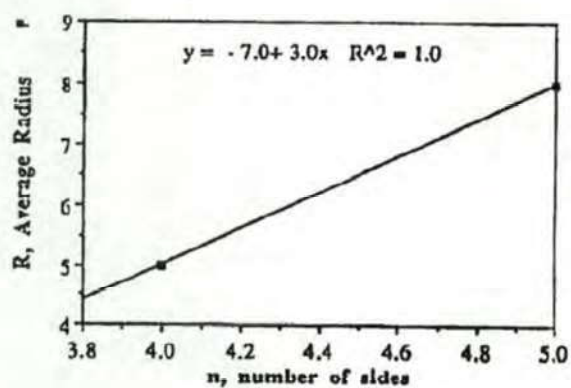


Figure 4

Average radius as a function of the number of sides for each n sided cell in the: a) Control web, b) Marijuana web, c) Benzedrine web, d) Caffeine web, and e) Chloral hydrate web.

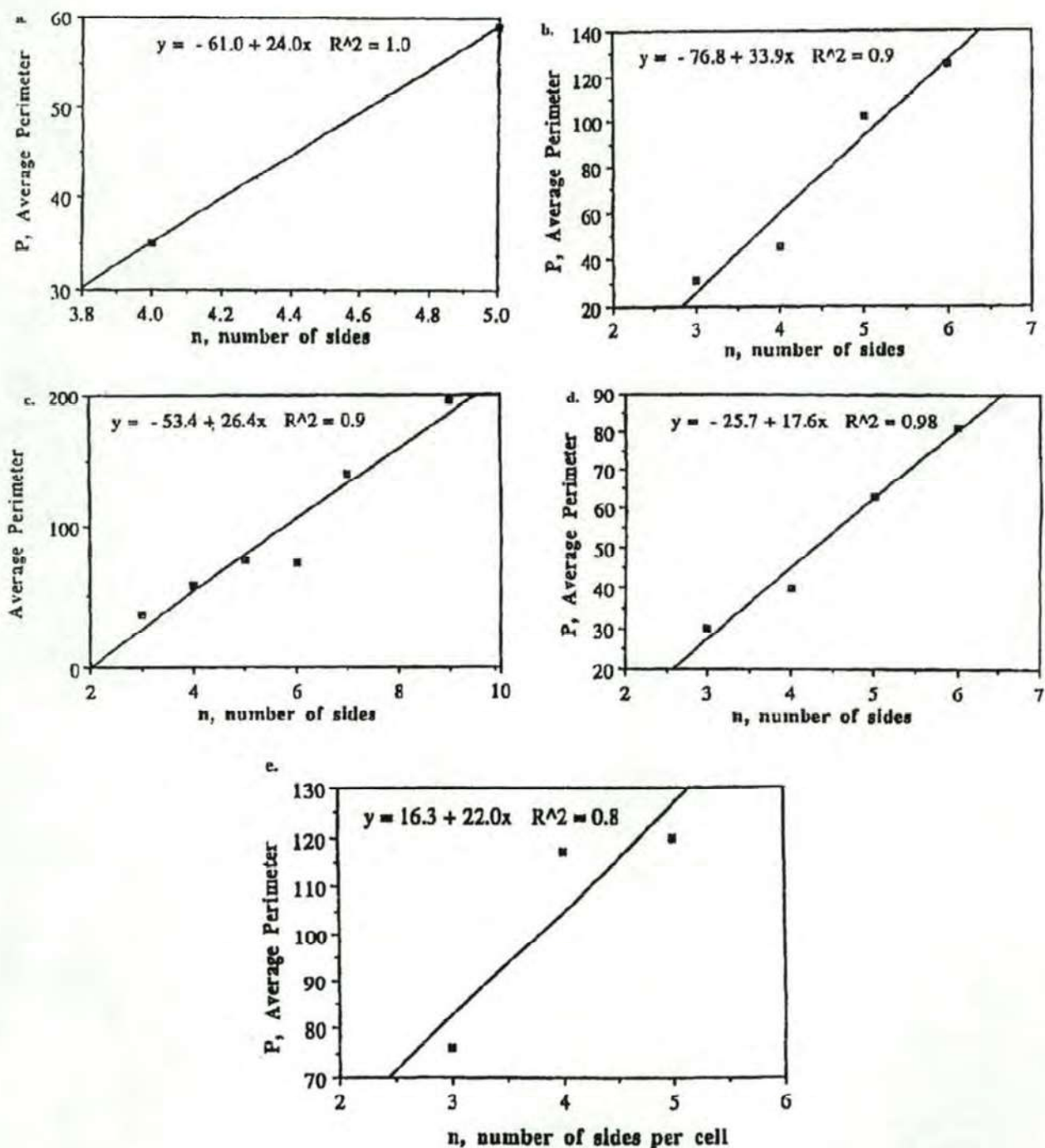


Figure 5

Average perimeter as a function of the number of sides for each n sided cell in the: a) Control web, b) Marijuana web, c) Benzedrine web, d) Caffeine web, and e) Chloral hydrate web.

Table 1

Toxicity of the drugs in spiders measured by judging which sides are complete. The least toxic web (control) has all sides completed. The most toxic web (chloral hydrate) does not have any sides completed. This system can be checked by the fact that the control web has not been affected by a drug, and it fits the least toxic classification.

<u>Web Type</u>	<u>Up</u>	<u>Down</u>	<u>Left</u>	<u>Right</u>
Control	C	C	C	C
Marijuana	NC	C	C	C
Benzedrine	C	C	NC	NC
Caffeine	NC	C	NC	NC
Chloral Hydrate	NC	NC	NC	NC

C = Completion of Web Side
NC = Failure to Complete Web Side
Least Toxic = No NCs, All Cs
Most Toxic = All NCs, No Cs

The least toxic drug, according to this classification system, has all four sides completed. The most toxic drug has none of the four sides completed. The five webs taken happen to represent each of the five categories: four sides completed, three sides completed, two sides completed, one side completed, and no sides completed. This method is based on the fact that a toxic drug will interfere with web formation. The rank order of toxicity in spiders is different than that of rats given the same chemicals. Figure 6 shows that marijuana seems to have little effect on spiders while it is the most toxic drug of the five for rats.

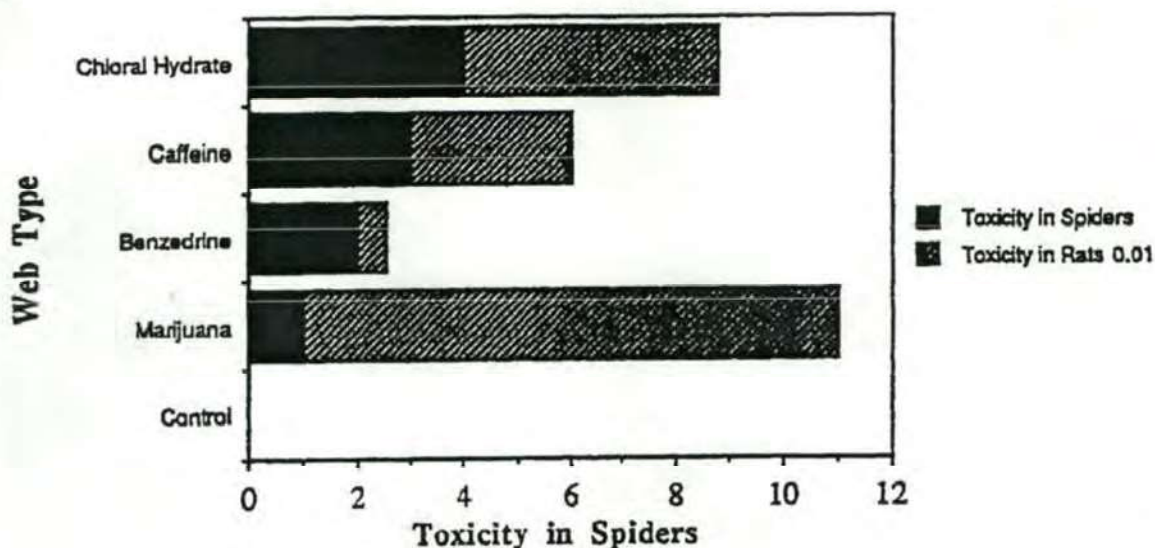


Figure 6

The toxicity of control, marijuana, benzedrine, caffeine, and chloral hydrate in rats versus their toxicity in spiders.

V. CONCLUSIONS

We investigated five different spider webs and from tracings of their webs we measured distributions concerning the shape and size of the cells. The webs are treated using powerful analysis techniques applied previously to random, 2-D networks¹. We found that:

1. All five spider webs adhere to Lewis's Law, with the average area increasing with the number of cell sides.
2. Four of the webs also followed a linear relationship with the average radius and number of sides ($R^2 > 0.9$). Chloral hydrate has a much lower correlation (0.6).
3. All five webs adhere to the Perimeter Law. The control, marijuana, benzedrine, and caffeine webs show a better correlation than the chloral hydrate web.
4. The chloral hydrate web shows a less accurate correlation in both Figures 4 and 5. This could be because the web was never completed; the effects of the drug became too disabling for the spider.

5. The distribution of n -sided cells does not match Euler's ideal of perfect hexagons which fill space without leaving any gaps (honeycomb patterns).
6. Toxicity of a drug on a spider can be determined by placing the web in one of five classifications depending on the number of completed sides of the web.

It is concluded that average area and average perimeter are correlated with the number of sides n for a given closed polygon within a spider web.

Spider webs are an example of two dimensional random cellular structures that present the general geometrical features of membrane networks. By connecting a biological pattern (webs) to other networks (soaps, monolayers, metal grains), then a more universal form for discussing their origin is initiated.

Future work should:

1. Enlarge the scope of drugs applied to include representative examples of carcinogens, insecticides, fungicides, petroleum products and organics, antimetabolites, and heavy metals.
2. Quantify the delayed effects of drug applications to spinning time, recovery period, as well as looking for generational effects on offspring webs.
3. Differentiate between methods of drug delivery (gas, liquid injection, dosages).
4. Magnify the web-spinning area to include more total number of closed cells, while still excluding the boundary cells and the large central cell; a larger sample size will prove advantageous to a drug testing plan. The present, more modest approach sets the methodology for these investigations. The sensitivity of spiders to drugs appears in their next web, which exhibits unique properties due to the drug. This leads to a mathematical framework to consider the possibility of using spiders and their webs to test drugs. This study provides some simple quantitative ways with which to compare the webs, both among each other and between a drug-altered and a control web.

VI. ACKNOWLEDGMENTS

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